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# **MEMBRANE SEPARATION**

## Jerry Lundstrom

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Instrumentation Research Laboratory, Department of Genetics Stanford University School of Medicine Palo Alto, California

## MEMBRANE SEPARATION

## Prepared by Jerry Lundstrom

Technical Report No. IRL-1046

April, 1966 Revised Edition

## Prepared under

## NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Grant NsG 81-60

"Cytochemical Studies of Planetary Microorganisms:

Explorations in Exobiology'

Principal Investigator: J. Lederberg
Program Director: E. Levinthal

Instrumentation Research Laboratory, Department of Genetics

Stanford University School of Medicine

Palo Alto, California

## 1. INTRODUCTION

The general approach and some of the basic mathematical relations of the membrane separation study of small enzymatic breakdown products from larger substrate molecules have been developed in a previous report (1). Since that report, particular emphasis has been directed at making permeability measurements that would be relevant to the separation of CO<sub>2</sub> from glucose.

Knowing the permeability coefficients of CO<sub>2</sub> and glucose for a given membrane, one can estimate the minimum metabolic rate for the conversion of glucose to CO<sub>2</sub> that is detectable using carbon-14 labelled glucose. This metabolic rate can then be equated with CO<sub>2</sub> production rates from glucose for various microorganisms to gain some insight into the sensitivity of the membrane separation device in terms of detectable numbers of microorganisms. These detectability estimates in numbers of Escherichia Coli for different values of the experimental parameters of observation time and membrane thickness have been worked out and are included in the Detectability Estimate section (IV). As an introduction to some of the results of section IV and as an illustration of an approximate approach to the analysis of a membrane separation problem, the separation factor is discussed briefly in the next few paragraphs.

The separation factor is the ratio of the steady-state permeability coefficients of the two solutes of interest, e.g.  $K_{CO_2}/K_{glucose}$ , for a

given membrane (in the  $\mathrm{CO}_2$ -glucose case, we have assumed that the maximum integral coefficient approximates the steady-state coefficient for glucose). By this definition it is also the ratio of the upstream concentration of glucose to  $\mathrm{CO}_2$  required for the permeability rates of the two solutes across the membrane to be equal. The primary value of the separation factor, aside from its being a convenient form for expressing the permeability data of a pair of solutes for a given membrane, lies in its use as a gross comparison between different membrane systems of their effectiveness in separating a given pair of solutes. To illustrate this use we compare the separation factors of the  $\mathrm{CO}_2$ -glucose pair for the membranes teflon (SF<sub>1</sub>) and silicone rubber (SF<sub>2</sub>) (necessary experimental data are given in sections II and III) with the separation factor (SF<sub>3</sub>) predicted previously<sup>(1)</sup> for silicone rubber from the published gas phase  $\mathrm{CO}_2$  permeability coefficient and the dye permeability coefficient measured in some preliminary experiments in this laboratory. The value are:

$$SF_1 = K_1/K_1' = C_1'/C_1 = 5 \times 10^6$$
  
 $SF_2 = 5 \times 10^5$   
 $SF_3 = 2 \times 10^9$ 

where:

 $K = the CO_p$  permeability coefficient

 $K^{T}$  = the glucose permeability coefficient

 $C_1$  = the upstream  $CO_2$  concentration for teflon membrane

 $C_1^{\tau}$  = the upstream glucose concentration for teflon membrane

From these values one would predict that the teflon system is about ten

times more effective in its separation of  ${\rm CO}_2$  from glucose than the silicone rubber system and that the third system is about 4000 times more effective. These predictions are only valid when the concentrations are independent of time.

In applications where one of the solute's concentration changes with time, e.g., applications where the concentration drops appreciably due to diffusion out of the solution vessel or where the solute is being produced or consumed at an appreciable rate, the factors affecting the solute's concentration may change the effectiveness of the membrane separation and complicate the analyses of a given membrane in a given separation application. In the Multivator application  ${\tt CO}_2$  is being produced at a constant rate by the microorganisms in a glucose solution (glucose is also being consumed but at a negligible rate compared to the total amount present). We have discussed these factors at some length in Section IV.

The permeability measurements and a brief discussion of these results along with a description of the experimental apparatus and method of analysis for glucose and  $\mathrm{CO}_2$  can be found in the Glucose Permeability (III) and the  $\mathrm{CO}_2$  Permeability (III) Sections. In the final section, Detectability Estimates, the results of Sections II and III are applied to the life detection problem. The mathematics for estimating the detectability of a known microorganism, e.g., E. Coli, in the life detection device and for interpreting the experimental results of the life detection device for an unknown microorganism have been developed. Both constant and multiplying populations are considered.

## 11. GLUCOSE PERMEABILITY

## A. Experimental

The sensitivity of the method as a detector of microorganisms is such that the glucose permeability experiments must be conducted under sterile conditions. The conditions found to be adequate to give reproducible permeability results and an absence of growth in a thioglycollate medium were:

i) exposure of the permeability cell and the membrane to an autoclave temperature of 124°C for a period of two hours, and 2) careful handling of the cell and membrane after the autoclave exposure in mounting the membrane and in filling the chambers of the permeability cell with the sterile solutions. The main concern in the latter condition was to prevent the upstream chamber from being separated from the membrane and thereby allowing the upstream chamber to possibly be contaminated by air-borne microorganisms.

The most extensive glucose permeability measurements have been made using silicone rubber membranes. The sterility of the upstream and downstream solutions in all these experiments has been verified by the absence of growth after incubation at 37°C for periods greater than ten days in a sterile thioglycollate medium. The medium was inoculated with a few tenths of a mi of the solution extracted at the end of the experiment. None of these experiments showed growth in the sterile medium.

Departure from the normal sterilization techniques in the case of porous glass was necessary in order that the porous glass would not be fractured

by the sudden temperature changes of the autoclave. The procedure consisted of boiling the porous glass disc in 30%  $\rm H_2O_2$  for about fifteen minutes. This treatment also removed absorbed organic vapors from the porous glass to the extent that the color of the porous glass changed from yellow to translucent. The 30%  $\rm H_2O_2$  treatment was followed by rinsing the porous glass in concentrated chromic acid and then soaking it in dilute  $\rm (NH_4)_2Fe$   $\rm (SO_4)_2$  solution. The  $\rm (NH_4)_2Fe$   $\rm (SO_4)_2$  treatment reduced any chromic acid and  $\rm H_2O_2$  remaining in the porous glass. The permeability cell was also rinsed in concentrated chromic acid. The final treatment of the cell and the porous glass prior to the experiment was to allow them to stand in sterilized distilled  $\rm H_2O$  for several hours. This modified sterilization procedure was assumed to be sufficient for the relatively short duration of the permeability experiment (a few hours).

The permeability cells used in the glucose measurements were one of two types, either the 100 cm<sup>2</sup> area stainless steel (No. 304) cell or the 10 cm<sup>2</sup> area Al cells used in the previously reported dye permeability measurements<sup>(1)</sup>. A photograph of the permeability cells is given in Figure 1. In the case of the Al permeability cells the geometry and orientation of the cell was such that there existed a possibility of an air bubble being trapped in the cell during the filling of the chambers with the solutions. For this reason the back walls of both the chambers in the cell were made of glass discs, thereby making it possible to see and to eliminate any air that was trapped in the chambers during their filling. In order that the glass discs would not be broken and that the chamber would not leak after the cell was clamped together a disc of teflon orpolyethylene was placed on

both sides of the glass discs before the cell was clamped together. Fresh polymer discs were used in each experiment. In the stainless steel cells the orientation and geometry of the cell were different and the possibility of trapped air was not a problem. The downstream chamber of the stainless steel cell was filled first and then the membrane placed on top of the downstream chamber. The amount of solution put into the upstream chamber after the cell was clamped together was ample to cover the top surface of the membrane.

We found that a slight bevel to either or both of the cell faces in contact with the membrane gave a better metal-membrane-metal seal and diminished the possibility of the solute diffusing around the membrane. Angles of about  $\mathbf{1}^{\mathbf{0}}$  appeared to be sufficient for a good seal as indicated by the reproducible permeability results for a given solute-membrane pair and by the well-defined disc of the dye disodium fluorescein (NapFI) dissolved in the cellophane membrane at the end of a NapFl experiment. The amount of NapFl in the hydrophobic membranes is much less than in cellophane so that one could not see any of the NapFl in the hydrophobic membranes and hence could not distinguish a disc corresponding to the exposed area. In the case of the hydrophobic membranes, however, no change was observed for different exposed areas, i.e. 100 cm<sup>2</sup> or 10 cm<sup>2</sup>. A change in the exposed area would change the ratio of the area to the perimeter by a factor r/2 and also change the observed permeability if the solute were diffusing around the membrane at the perimeter of the exposed area instead of through the membrane.

One might be able to verify that the permeation was occurring through only 6.

the defined area by using a radioactive solute. That is, after the permeability experiment was complete, the membrane could be washed superficially and then cut into small squares of equal area. Each of the squares could be ground into a fine powder and then suspended in a small volume of a concentrated glucose solution to equilibrate the labelled glucose in the membrane with the glucose in the water phase. After standing, the water phase could then be separated from the membrane particles by filtration and transferred to a scintillation solution for counting. All of the squares from the center of the disc should have the same activity and the average of several of these could be used to determine the activity per unit area. From the total activity of all the squares, one could then calculate the actual area that the solute permeated through. A more convenient choice of membrane might be found for this experiment, i.e. one that will dissolve in the organic solvent used for the scintillation solution.

The assays used to measure the amount of glucose that had diffused through the membrane after a given time have been described in a previous report (1). We have not attempted the counting of aqueous glucose solutions directly but have thus far only used non-aqueous scintillation solutions in which a small volume of an aqueous solution will dissolve or in which the oxidized product of glucose, i.e. (0), will be trapped.

The sources of error in the measurement of the glucose permeability coefficients are:

1) The error in the upstream concentration of glucose. Prior to each experiment (and in some cases, also after the experiment was completed)

a sample of the upstream solution was counted by the same technique to be used in counting the downstream solutions during the experiment (for the  $100 \text{ cm}^2$  area cell-oxidation of the glucose to  $\text{CO}_2$  with conc.  $\text{H}_2\text{SO}_4$  and  $\text{CrO}_3$  saturated solution and simultaneous trapping of the  $\text{CO}_2$  in a hyamine-toluene solution with subsequent liquid scintillation counting of the hyamine-toluene solution; for the  $10 \text{ cm}^2$  area cells-dissolution of the glucose solution in a scintillation solution using dimethoxyethane as the solvent with subsequent counting of the dimethoxyethane solution).

Since the same technique for counting the upstream solution was used in counting the downstream solutions, the systematic errors in the technique did not appear as errors in the permeability coefficients. The random errors of the volume measurements and the number of cpm\*in the sample, however, must be considered. For the upstream solution the error from volume measurements was less than 5% and the counting error was less than 1%. The total relative error in the glucose upstream concentration was, therefore, less than 6% (relative to the glucose counted in the downstream solutions).

2) The error in the amount of glucose in the downstream solutions. For the experiments carried out in the  $100~{\rm cm}^2$  area cell the entire  $25\pm.5{\rm ml}$  contents of the glass vial (total downstream volume less the small volume in contact with the lower surface of the membrane) were quantitatively transferred to a flask for the glucose oxidation and the trapping of  ${\rm CO}_2$ . The error in the volume measurement is less than 2%. The hyamine-toluene solutions were counted to an error of less than 1%. The total error in

\*Unit of observed radioactivity: cpm = counts per minute.

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each of the downstream samples from the 100 cm<sup>2</sup> cell is less than 3%.

For the experiments carried out in the 10 cm<sup>2</sup> area cells, the solutions were counted to an error of less than 3%. The volume of the solution counted was measured with a special Hamilton syringe that was reproducible to 1% for a volume of 1 ml. In the ideal case the total downstream volume of the 10 cm<sup>2</sup> area cell would be constant for the entire experiment and the precision of the volume measurements would then be 1%. In the actual case, however, the total downstream volume varied by a few tenths of a ml. during the experiment so that the highly reproducible 1 ml. volume extracted represented a changing fraction of the total downstream volume (a typical volume is 1.5 ml). The error in the downstream volume measurement is less than 5%. The total error in the glucose content of a downstream sample for the 10 cm<sup>2</sup> cell was, therefore, less than 8% (5% for the volume and 3% for the counting).

- 5) The error in the membrane thickness is less than 5% for silicone rubber and less than 20% for the thinner teflon membrane.
- 4) The error in the temperature of the system is less than 1%.
- 5) The error in the membrane area is less than 1%.

The maximum estimated error in the glucose permeability coefficients for the experimental systems described is about 20% for the silicone rubber experiments and about 40% for the teflon experiments. Additional deviations that may arise from different membrane samples have not been considered here.

#### B. Results

The permeability results of glucose through silicone rubber membranes are summarized in Table 1. Some permeability data have been gathered for glucose through other barriers, i.e. teflon and porous glass. These data are given in Table 2.

The glucose permeability curve has a different shape than expected for a permeability process limited exclusively by the solute's diffusion through the membrane. The typical permeability curve where the permeability is diffusion limited has been described in a previous report (1). The permeability curve for glucose through silicone rubber is characterized by an "initially rapid" permeability rate that reaches a maximum and then decreases to some slower rate with increasing time of the experiment. Only slight differences\*in the shape of the permeability curve were observed for changes in the glucose upstream concentration over a 10<sup>4</sup>-fold range (see Table 1). The concentration of carbon-14 in the upstream chamber was maintained constant for these changes in glucose concentration.

On a number of occasions samples of the upstream solution of glucose were extracted from the permeability cell at the end of an experiment and chromatographed on Whatman No. I paper using n-butanol saturated with water and NH<sub>14</sub>HCO<sub>3</sub> as the solvent. Although the fraction varied for different samples, in every case some of the carbon-14 label remained at the base line of the chromatogram (an amount in excess of the base line fraction for the standard solution of glucose). The remainder of the label coincided approximately with the peak observed for a dilute stock solution of carbon-14

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<sup>\*</sup>More noticeable differences began to appear after about  $5 \times 10^5$  seconds that seemed to be roughly correlated with the upstream concentration, i.e. the lower the upstream concentration the higher the apparent long-time permeability.

labelled glucose. The dilute stock solution of glucose was prepared by a hundred fold dilution of the same glucose stock solution used in the permeability experiments. This dilution was made the same day the glucose stock solution was prepared from the solid glucose stock. The dilute glucose stock solution was stored in a sterile bottle and a lyophilized sample of this solution was used as a reference for each paper chromatographic analysis. The dilute solution was lyophilized so that a ten microliter spot of the reference would contain about the same amount of carbon-14 as the ten microliter samples extracted from the upstream solutions of the permeability cell.

Whether these solutions were taken from the stainless steel or Al cells did not appear to matter since samples from both cells had a fraction of the label at the base line that exceeded the base line fraction of the glucose reference solution. In most of the samples the base line fraction was small. For the exceptional case plotted in figure 2\*, however, the base line fraction exceeded the fraction appearing at the glucose peak.

Since the chromatogram was analyzed by cutting I cm wide strips parallel to the base line (and then counting these strips in a dioxane-toluene scintillation solution), a point on the curve represents the cpm located

<sup>\*</sup> The solution of curve B was filtered through a bacterial millipore filter prior to the chromatographic analysis. The solution of curve C was taken from the upstream chamber and chromatographed directly without filtering. The cpm given in the legend of figure 2 after the description of each of the curves is the total number of cpm above background for that curve.

on the paper between the distance corresponding to the given point and the distance of the previous point (the previous point is the adjacent point having the shorter distance). For example, the fraction of the carbon-14 located on the paper between the base line and 1 cm from the base line corresponds to the point 1 cm from the base line in fig. 2. This method of analysis is limited to a gross comparison of the curves. For example, one cannot decide if the fractions of curves B and C located between the base line and 2 cm occur at the same distance from the base line or not, since the fraction between 1 cm and 2 cm for curve B could be anywhere between 1 cm and 2 cm, inclduing the possibility of the fraction being right at the 1 cm edge. The method is adequate, however, to indicate that a large fraction of the carbon-14 remained near the base line for both curves B and C, and that the remainder of the carbon-14 approximately coincided with the glucose peak of curve A.

The upstream sample of curves B and C was exposed to a membrane\* for about two months in the 100 cm<sup>2</sup> cell. This is about twice as long as the exposure of the other upstream samples chromatographed and it is possible that this might explain the exceptionally large base line fractions shown in curves B and C. This, of course, does not account for the occurrence of a base line fraction in excess of that for the standard glucose solution.

<sup>\*</sup>The membrane was changed from a teflon membrane to a silicone rubber membrane after about one month. The chromatographic analysis after the first month produced results similar to those observed for the other samples chromatographed and described above, i.e. the base line fraction was small and the bulk of the carbon-14 approximately coincided with the glucose peak.

#### C. Summary

The permeability results of glucose indicated that the solute-membrane interaction is important in the glucose transport across silicone rubber since the glucose-silicone rubber permeability curve is of a different general shape, i.e. "initial rapid" increase in the permeability that appears to decrease with time, than the typical permeability curve where the transport is limited by the diffusion process exclusively. In addition, some paper chromatographic studies of the upstream glucose solutions indicate that some change in the solutions had occurred while the solutions were in contact with the membrane.

For the purposes of determining the feasibility of the separation of  $\mathrm{CO}_2$  from glucose by the use of a teflon or silicone rubber membrane, a fundamental understanding of the glucose transport through the membrane is probably not so important as knowing the actual permeability coefficient of that transport. At least in the cases where the glucose is not oxidized to  $\mathrm{CO}_2$  by the glucose-membrane interaction\*. These permeability coefficients have been obtained for the barriers silicone rubber, teflon and porous glass, and are listed in tables 1 and 2.

<sup>\*</sup> The techniques for measuring the glucose transported across the membrane would not measure the amount of  $\rm CO_2$  produced in the membrane since in handling the downstream solutions no precaution was taken to limit the solution's exposure to the atmosphere and any carbon-14 labelled  $\rm CO_2$  would equilibrate with the  $\rm CO_2$  in the atmosphere. We have assumed that any carbon-14  $\rm CO_2$  produced in this way is negligible.

## 111. CO PERMEABILITY

## A. Experimental

The apparatus used for the  $CO_p$  experiments is pictured in fig. 3. It is made of stainless steel and is similar to the stainless steel cell used in the glucose measurements except that the membrane area for the  $co_{p}$  cell is  $10^{-1}$  cm<sup>2</sup> whereas the glucose cell's membrane area is 100 cm<sup>2</sup>. A small cup can be attached to the underside of the cap for the cell and is used to hold a small volume of acid (1M  $HNO_x$ ). The  $CO_p$  charge of about 10mg of carbon-14  $BaCO_3$  is held above the cup by a magnet taped to the outside of the permeability cell. The experiment starts when the magnet is removed allowing the  ${\tt BaCO}_{\bf x}$ , sandwiched between two pieces of filter paper, and its support (a polyethylene coated iron washer) to drop into the acid. The cup is separated from the upstream solution except for eight gas vents located near the top of the cup. Since the concentration of CO, in the upstream solution is dependent on the pH of the solution (particularly alkaline pH's) and on the pressure of  $CO_{\mathcal{P}}$  in the chamber it is an advantage to have the  $CO_{\mathcal{P}}$ source independent of the upstream solution. With an independent COp source one can control the amount of CO, that dissolves in the upstream solution for any given pH of the upstream solution. Having the CO source inside the chamber makes the permeability cell a self-contained unit that is convenient to use, e.g. during the experiment the cell is rotated at approximately 100rpm on a rotator but can be easily removed from the rotator for brief periods (less than 100 seconds) to sample 14.

the downstream solution.

For the  ${\rm CO}_2$  permeability coefficients reported here, the upstream solution (10ml) was buffered with  ${\rm 10}^{-2}{\rm M~H_2PO}_1$  at a pH = 2.2. The downstream solution (25ml) was buffered with  ${\rm 10}^{-2}{\rm M~Na}_2{\rm HPO}_1$  at a pH = 9.0. The higher pH in the downstream solution minimized the loss of  ${\rm CO}_2$  from the solution during the sampling period. After the downstream solution was removed for sampling it was replaced by a fresh solution.

A glass vial was used for the downstream chamber of the  $10^{-1} \text{cm}^2$  cell. This design allows the cell to be placed over a photomultiplier tube and with the use of a solid state scintillator, e.g. the Tracerlab scintillation beads, acqueous solutions can be counted directly. For the experiments described here, however, the downstream solution was removed periodically and the carbon-14 content was determined by the use of a nonaqueous liquid scintillation counting technique. This technique has been described in a previous report!

The concentration of  ${\rm CO}_2$  in the upstream chamber can be found from the published solubility coefficient of  ${\rm CO}_2$  in dilute acid solutions by assuming Henry's law and the Ideal Gas law.

Let X = M moles of  $CO_2$  produced from BaCO<sub>3</sub> Y = M moles of  $CO_2$  dissolved in the buffered solution  $S = \text{solubility of } CO_2$  in the buffered solution,  $\frac{m \text{ moles}}{ml. \text{ atm}}$   $V_1 = \text{volume of buffer solution, ml.}$   $V_2 = \text{free volume in the upstream chamber, ml.}$  $P_{CO_2} = \frac{(X-Y)RT}{V_2}$ 

Then: 
$$Y = S P_{CO_2} V_1 = \frac{SV_1(X-Y)RT}{V_2}$$

Rearranging: 
$$Y = X \left( \frac{SV}{V_2} \right)^{\frac{RT}{+}} SV_1 RT$$

The above expression for Y can be used to obtain the upstream  ${\rm CO_2}$  concentration. The  ${\rm CO_2}$  solubility in distilled water approximates the  ${\rm CO_2}$  solubility in a dilute acid solution and is .0344M/atm for dilute solutions of  ${\rm CO_2}$  at 25°C.

The sources of error in the measurement of the  ${\rm CO}_2$  permeability coefficients are:

- 1) The error in the number of m moles of  $CO_2$  in the upstream chamber Y, is estimated to be less than 10%. This includes the weighing error of  $BaCO_3$ , the error in the free volume of the upstream chamber and the error in the  $CO_2$  solubility.
- 2) The error in the membrane thickness is less than 5% for silicone rubber and less than 20% for the thinner teflon membrane.
- 3) The error in the temperature of the system is less than 1%.
- 4) The error in the membrane area is less than 1%.
- 5) The error in the counting efficiency of the transported CO<sub>2</sub> is estimated to be less than 35% for a 30% overall counting efficiency (i.e., the % counting efficiency could range from 20% to 40%).

A similar technique was used for counting  ${\rm CO}_2$  as was used for counting glucose where the glucose was oxidized and simultaneously trapped in a hyamine-toluene solution. From the glucose controls for the technique the % counting efficiency was found to be dependent on the shaking time. The % counting efficiency increased from about 21% at a shaking time of 16.

60 minutes to 30% at a shaking time of 100 minutes. After a 100 minutes the increase was less than an additional 10%. The % counting efficiency for  $\mathrm{CO}_2$  should have the same dependence since the  $\mathrm{CO}_2$  technique was the same as that for glucose so that the % counting efficiency for  $\mathrm{CO}_2$  at one hour is probably between 20% and 30%. We have assumed 30% as the % counting efficiency for  $\mathrm{CO}_2$  since 30% is the average % counting efficiency for shaking times greater than one hour and because 30% will give a more conservative estimate of the  $\mathrm{CO}_2$  permeability coefficient and the  $\mathrm{CO}_2$ -glucose separation factor than 20%.

The estimated maximum error is about 50% (about 70% for teflon) for the CO<sub>2</sub> permeability coefficients.

#### B. Results

The permeability data for  $CO_2$  through teflon and silicone rubber are plotted in fig. 4. The steady-state permeability coefficients can be calculated from the slopes of the respective permeability curves. The estimated maximum error in the coefficients is about  $\pm$  50%. The permeability coefficients are listed in Table 3. Other relevant diffusion and permeability coefficients for  $CO_2$  that are available from the literature have also been included in Table 3.

The permeability coefficients reported for  ${\rm CO_2}$  through teflon and silicone rubber for gas-membrane-gas systems (Table 3) are somewhat higher than those reported here for the water-membrane-water systems. The sorption of  ${\rm H_2}$  within the membrane in quantities sufficient to decrease the free space about the polymer strands in the membrane but markedly less than the amount needed to swell the membrane may account for the decrease in

the permeability coefficients observed for the water-membrane-water systems.

if the decrease in the coefficients is due to the water sorption by the membrane, then experiments carried out at higher temperatures would be expected to show better agreement between the  ${\rm CO_2}$  permeability in the two systems, i.e. in the water-membrane-water system and in the gasmembrane-gas system. The sorption of the  ${\rm H_2O}$  by the membrane is expected to occur to a lesser extent at the higher temperature and thereby would occupy "permanently" less of the free space in the membrane.

One experimental factor that is of concern is the alkaline pH of the downstream solution. It is conceivable that the  $\mathrm{CO}_2$  diffusion at or near the downstream surface of the membrane may be diminished if the  $\mathrm{CO}_2$  molecules react with the hydroxyl ions diffusing into this region from the downstream chamber. The resulting bicarbonate or carbonate ions are expected to have a smaller permeability coefficient than  $\mathrm{CO}_2$  and their formation would tend to decrease the observed permeability of  $\mathrm{CO}_2$ .

Since these  $\mathrm{CO}_2$  experiments were run, a calculation of the concentration distribution of  $\mathrm{CO}_2$  in the vial after the vial had been left open to the atmosphere for 100 seconds has been made. For an exposed surface of 2.5 cm<sup>2</sup> and a vial of 6 cm depth, less than 1% of the  $\mathrm{CO}_2$  had diffused out during the  $\mathrm{IO}^2$  seconds. This calculation assumed that the major species was  $\mathrm{CO}_2$  not  $\mathrm{HCO}_3^-$  or  $\mathrm{CO}_3^{-2}$  and that the concentration distribution of  $\mathrm{CO}_2$  was uniform initially (in the present experiments the loss of  $\mathrm{CO}_2$  is even smaller since  $\mathrm{CO}_2$  is a minor species in a solution of pH 9). An experiment

could therefore be conducted with both solutions at the same acidic pH (or neutral pH), thereby eliminating the troublesome experimental factor of the alkaline downstream solution.

#### C. Summary

The permeability coefficients of CO<sub>2</sub> through silicone rubber and teflon appear to decrease in a water-membrane-water system compared to the gasmembrane-gas system. This decrease is parallel for the two membranes in that the ratio of the CO<sub>2</sub> permeability coefficients for silicone rubber to teflon is about 10<sup>2</sup> for either a water-membrane-water system or a gasmembrane-gas system. The difference in the CO<sub>2</sub> permeability coefficient for a given membrane between the two systems, however, is greater than the maximum estimated error in the water-membrane-water results reported here and is now thought to be due to the decrease in free volume of the membrane occupied by a small quantity of water that is sorbed by the membrane. The differences in the permeability coefficients between the systems are expected to diminish with an increase in temperature if the sorption of water by the membrane is important.

In general our apparatus is quite serviceable for measuring the permeability coefficients of certain gases in a liquid-membrane-liquid system, e.g.  $CO_2$ ,  $H_2S$ ,  $SO_3$  and  $SO_2$ . The gases  $N_2$ ,  $NO_2$ ,  $NO_2$  and  $O_2$  could also be studied with the apparatus using methods other than radioisotope counting. One disadvantage, at least for the  $CO_2$  measurements, is that the equilibration time of the gas dissolution in the upstream solution appears to be too long and therefore prevents the measurement of the brief time lag of the

experiment and hence the calculation of the diffusion coefficient and of the solubility coefficient for the  ${\rm CO_2}$ -membrane system. Use of thicker membranes for  ${\rm CO_2}$  would be of some help, although the thickness is limited to some extent by the  ${\rm IO}^{-1}$  cm<sup>2</sup> area.

The water-membrane-water results for  ${\rm CO}_2$  given in Table 3 have been used in the final section for the estimates of the separation between  ${\rm CO}_2$  and glucose that can be expected for the two membranes, teflon and silicone rubber.

## IV. DETECTABILITY ESTIMATES

#### A. Introduction

Using the integral permeability coefficients for glucose through silicone rubber and teflon from tables I and 2 and the corresponding steady-state coefficients for  $\mathrm{CO}_2$  from table 3, one can estimate the separation factors for the two membranes. The separation factor as used here may be defined as the ratio of the concentrations of glucose to  $\mathrm{CO}_2$  in the upstream chamber required at steady-state for an equal number of glucose and  $\mathrm{CO}_2$  molecules to permeate through a given membrane per unit time. For silicone rubber the minimum separation factor is about 5  $\times$  10<sup>5</sup> for the period extending from about 6 to 30 hours after the start of the experiment (no permeability measurements have been obtained for glucose through silicone rubber for the period t = 0 to t = 6 hours). Using the best straight line value for the permeability coefficient of glucose the separation factor for teflon is about 5  $\times$  10<sup>6</sup> (the separation factor using the permeability coefficient for glucose at 7.7  $\times$  10<sup>1</sup> seconds is 10<sup>7</sup>).

In the detectability estimate made in the previous report (1) the estimated separation factor was about 2 X 10 and the questions asked were: For an experiment expected to run for 20 hours, what membrane thickness will be required to restrict the radioactivity from glucose diffusing into the detection chamber to 100 dpm? For this thickness how long will it take for .9 of the CO<sub>2</sub> produced in the upstream chamber to diffuse into the detection chamber? The detectable number of microorganisms would be the

\*Unit of theoretical radioactivity: dpm = disintegrations per minute.

number required for .9 of the  $CO_2$  produced to exceed the 100 dpm limit within the 20 hour period. These questions are no longer appropriate for the actual separation factors since for a thickness determined by the glucose permeability, the time needed for .9 of the  $CO_2$  produced in the upstream chamber to diffuse into the detection chamber exceeds the time of the experiment.

The simplest modification to the series of questions would be to ask for the time needed for some smaller fraction than .9 to diffuse into the detection chamber. In the case of a silicone rubber barrier no useful result is obtained since the membrane thickness necessary to restrict the glucose level in the downstream chamber to 100 dpm is found to be about 15 cm for a 20 hour experiment. For a teflon barrier, however, the membrane thickness is about  $1.5 \times 10^{-2}$  cm and the corresponding time for steady-state  $co_2$  permeation is about  $10^3$  seconds. These results are comparable to those predicted from the published CO<sub>2</sub> data and our dye permeability measurements (1) (except that the time for steady-state is about 400 times greater than predicted since the predicted results used the  ${\it CO}_2$ -silicone rubber gas permeability data). Using the actual teflon data, for an experiment lasting about 20 hours one might expect to detect as few as 20 - 30 microorganisms. This compares favorably with the 10 microorganisms ( nonmultiplying) predicted in our previous report (1) for a 20 hour experiment. The calculations used here are similar to those given in the previous report except that instead of the .9 of the  ${\rm CO}_{\rm p}$  produced in the upstream chamber diffusing into the downstream chamber, only .4 of the  ${\rm CO}_2$  produced has diffused into the downstream chamber.

The more appropriate series of questions for the experimental separation factors that allows us to make some quantitative statements about both the silicone rubber and teflon systems is: For a given membrane thickness x and number of microorganisms n, how long must one wait for the ratio of  $\mathrm{CO}_2$  to glucose in the detection chamber to reach some selected value, e.g. 6 or 60, with the restriction that the total radioactivity in the detection chamber exceeds a minimum noise level such as 100 dpm? Considering this time does the membrane thickness x satisfy the condition that the time for steady-state  $\mathrm{CO}_2$  permeation is equal to or less than 1% of the time required for the given ratio of  $\mathrm{CO}_2$  to glucose to have diffused into the detection chamber? In the case that x does not satisfy this condition a smaller value of x can be tried. As will be seen later in this section, the device is most efficient when t and x just satisfy this condition, i.e., t equals 100T (T equals time for steady-state).

By imposing this condition we can treat the  ${\rm CO}_2$  transport as a steady-state transport and avoid solving the more exact and more difficult non-steady-state problem. The choice of 1% for the steady-state condition is somewhat arbitrary and one might choose a less restrictive percentage and obtain a more approximate representation of the  ${\rm CO}_2$  transport, e.g. 10%. An estimate of the error in the approximate solution of the problem can be obtained by holding the upstream concentration constant after some time t and allowing the  ${\rm CO}_2$  transport to continue until it reaches steady-state permeation. The difference in the number of molecules of  ${\rm CO}_2$  permeating under steady-state conditions and the actual number of molecules of  ${\rm CO}_2$  permeating for the interval is an estimate of the error

in the approximate solution of the  ${\rm CO}_2$  transport problem. The maximum error is to overestimate the amount of  ${\rm CO}_2$  transported by nmT (for any system, the maximum permeability rate is equal to the rate of  ${\rm CO}_2$  production, i.e. nm, and T is the time for steady-state). For the 20 hour experiment where n = 25 microorganisms, m = 2.4 x  ${\rm 10}^9$  molecules/cell hour  $^{(4)}$  and  ${\rm T}_{10\%}$  = 2 hours or  ${\rm T}_{1\%}$  = .2 hours, the maximum errors for the two cases are  ${\rm E}_{10\%}$  = 1.2 x  ${\rm 10}^{11}$  molecules and  ${\rm E}_{1\%}$  = 1.2 x  ${\rm 10}^{10}$  molecules. Comparing E1% and  ${\rm E}_{10\%}$  with the minimum number of  ${\rm CO}_2$  molecules that are detectable, i.e. 4.2 x  ${\rm 10}^{11}$  molecules equals 100 dpm, an overestimation of E1% in the problem is negligible whereas  ${\rm E}_{10\%}$  is within a factor of four of the detectability threshold and is not a negligible overestimation. Hence our 1% steady-state condition would provide a reasonably accurate representation of the  ${\rm CO}_2$  transport whereas the 10% condition might not.

## B. Constant Populations of Microorganisms

in order to derive a general expression for the ratio of  $\mathrm{CO}_2$  to glucose in the detection chamber at time t for a system where the concentration of  $\mathrm{CO}_2$  in the upstream chamber is a function of time, one can first derive an expression for the concentration of  $\mathrm{CO}_2$  in the upstream chamber for the case where no diffusion takes place and then for the case where diffusion out of the chamber occurs. The difference between these two concentrations times the upstream chamber volume V is the amount that has diffused out of the chamber during time t.

For n microorganisms producing  $CO_2$  at a rate m in a chamber of volume V, the concentration of  $CO_2$  in the chamber after t seconds, C\*(t), assuming  $CV_2$ .

no  ${\tt CO}_2$  diffuses out of the chamber and that n is constant over the time t is:

(6) 
$$C*(t) = \frac{nmt}{V}$$

If the chamber has for one surface a membrane of area A and thickness x with a permeability coefficient for  ${\rm CO_2}$ , K, the change in the number of molecules of  ${\rm CO_2}$  in the chamber during the infinitesimal time interval, dt, is:

(7) 
$$VdC = nmdt - \frac{KAC}{x} dt$$

(8) 
$$\frac{dC}{dt} + \frac{KA}{Vx}C = \frac{nm}{V}$$
Multiplying by the integrating factor,  $e^{\frac{AK}{Vx}}t$ 

(9) 
$$\frac{\frac{AK}{Vx} t}{dt} = \frac{nm}{V} e^{\frac{AK}{Vx} t}$$

Multiplying by dt and integrating both sides,

(10) 
$$Ce^{\frac{AK}{Vx}t} = \frac{nm}{V} \frac{Vx}{AK} e^{\frac{AK}{Vx}t} + constant$$

From the initial conditions: t = 0, C = 0 one can evaluate the constant.

$$0 = \frac{mnx}{AK} + constant$$

With substitution for the constant and rearrangement, (10) becomes:

(11) 
$$C(t) = \frac{mnx}{AK} (1 - e^{-\frac{AK}{Vx}} t)$$

Equation (11) gives the concentration of  $\mathrm{CO}_2$ ,  $\mathrm{C}(t)$ , in the upstream chamber at time t. As t increases the exponential term in (11) decreases and  $\mathrm{C}(t)$  approaches  $\frac{\mathrm{mnx}}{\mathrm{AK}}$  asymptotically. The physical interpretation of this upper limit to  $\mathrm{C}(t)$  is that the rate of diffusion of  $\mathrm{CO}_2$  out of the chamber equals the rate of production of  $\mathrm{CO}_2$  in the chamber for  $\mathrm{C}(t)$  equal to  $\frac{\mathrm{mnx}}{\mathrm{AK}}$ .

Assuming that the amount of  ${\rm CO}_2$  dissolved in the membrane and adsorbed to the inner surface of the two chambers is negligible, the number of molecules of  ${\rm CO}_2$  that has diffused into the detection chamber at time t is the difference between the number of molecules produced in time t (equation (6)) and the number of molecules remaining in the upstream chamber at time t (equation (11)).

An upper limit on the number of glucose molecules that have diffused into the detection chamber, q', during time t can be found from the integral permeability coefficient, K'. Using the steady-state permeability q' as given by Fick's first law:

(12) 
$$q' = \frac{AtK'C'}{x}$$

where the primed quantities refer to glucose,

C' = the upstream concentration of glucose and is practically constant for t<10° seconds.

K' = integral permeability coefficient for glucose

The ratio of  $CO_2$  to glucose molecules that have diffused into the detection chamber, r(t,x,n), can be obtained from equation (6), (11), and (12).

(13) 
$$r(t,x,n) = \frac{v(c*(t) - c(t))}{q^i}$$

$$= \frac{xmnt - \frac{mnx^2}{AK}}{AtK'C'} (1 - e^{\frac{-AK}{XV}}t)$$

The derivation of the expression r(t,x,n) for the ratio of  $co_2$  to glucose in the detection chamber assumes that : 1) The downstream concentration

<sup>\*</sup>As in the previous report, C' was assumed to be  $10^{-3}$  molar with all the carbon atoms in glucose assumed to be carbon-14.

is negligible with respect to the upstream concentration for all t > 0.

- 2) The upstream concentration is uniform for the entire experiment.
- 3) The amount of CO<sub>2</sub> and glucose dissolved in the membrane and adsorbed on the inner surfaces of the two chambers is negligible with respect to the quantities diffusing into the downstream chamber.

As t increases r(t,x,n) approaches an asymptote; if the upper limit of r is called  $r_{max}$  then (13) can be written as:

(14) 
$$r = r_{\text{max}} \left[ 1 - \frac{Vx}{KAt} \left( 1 - e^{\frac{-AKt}{XV}} \right) \right]$$
where

$$r_{\text{max}} = \frac{mnx}{AK^{\dagger}C^{\dagger}}$$

From equation (15) one notes that the maximum value of r for a given physical system is proportional to the number of microorganisms present, n. If one chooses a value of t, then x can be found from the condition that the time for steady-state  $c_2$  permeation be short compared with t, e.g. 1% of t. The time for steady-state, t, can be estimated from t

(16) T **≃** 
$$\frac{x^2}{2K}$$

By substituting the values of t and x into (14), one can find the relationship between r and  $r_{max}$ . Then by choosing a value of r, n can be found from (15). This n represents the number of microorganisms required to achieve the desired ratio, r(t,x,n), of  $co_2$  molecules to glucose molecules in the downstream chamber at a given time t for a given membrane thickness  $x_1$ .

The choice of it is regulated by two criteria: 1) r must be compatible with the limitations of the detector measuring the amount of radio-activity present. The relationship between r and the total disintegration rate in the downstream chamber (referred to as the total number of dpm) is given by (17).

(17) total number of dpm = 
$$Hq'(r+6)$$

where q' = the number of glucose molecules in the downstream chamber at time t.

H = the conversion factor for atoms to dpm assuming all the carbon in glucose is carbon-14.

=  $2.4 \times 10^{-10}$  dpm/carbon atom

The 6 in (17) accounts for the 6 carbon atoms per glucose molecule and requires that r=6 for the  $\operatorname{CO}_2$  signal to be just equal to the glucose signal. 2) In addition, the choice of r must take into account the reproducibility of the permeability coefficient for various samples of a given kind of membrane. Choosing r=60 readily satisfies these two criteria, i.e. that the total number of dpm in the detection chamber exceeds the 100 dpm noise level of the detector and r is large compared to the reproducibility of the maximum glucose permeability coefficient. The value r=60 has been used in the subsequent detectability calculations.

In figures 5 and 6 log n is plotted versus log t for different values of x. An upper bound is placed on x so that 100T is not greater than  $10^6$  28.

seconds. For the curves extending over the entire time range, t equal to 100 T is less than  $10^3$  seconds. For the other curves the smallest value of t plotted is equal to 100 T. A and V used in the calculations were taken as  $1 \text{ cm}^2$  and  $1 \text{ cm}^3$ . The value of m used was obtained from Clifton's data for  $C0_2$  production from glucose by Escherichia Coli. (4)

In figures 5 and 6 the number of bacteria, n, required for r = 60 decreases with increasing time t and with increasing membrane thickness x. For a given x, the decrease in n with an increase in t is due to the COp permeability rate approaching the maximum CO<sub>p</sub> permeability rate, i.e. the rate of production of  ${\rm CO}_{\rm p}$  in the upstream chamber. For a given t, the decrease in n with an increase in x is more complicated. First, the max $imum CO_{2}$  permeability rate is independent of x and given enough time the CO, permeability rate will equal the rate of production of CO, regardless of the value of  $\mathbf{x}$ . The  $\mathrm{CO}_{\mathrm{p}}$  permeability rate at some time less than the time needed for the maximum CO, rate will, however, be decreased by an increase in x. The relation between this  $CO_p$  rate and x is nearly inversely proportional at times much less than the time required for the maximum  $CO_p$  rate. The  $CO_p$  rate becomes more independent of x as the time increases. Since the glucose rate is inversely proportional to x (equation 17), the overall dependence of r(t,x,n) on x varies from nearly independent of x at times much less than the time required for the maximum  $CO_p$  rate to proportional to x at the time required for the maximum CO, rate (equation 14). From equations (14) and (15) n is proportional to r for all t so for r(t,x,n) set equal to a constant, n is nearly independent of x at times much less than the time required for the maximum  ${\rm CO}_{\rm p}$  rate and becomes inversely proportional to x at the

time required for the maximum CO, rate. These extremes of the relation between n and x for a given t can be seen in fig. 6. For example, the value of n decreases only slightly when the thickness is changed by a factor of 2 from 5 X  $10^{-3}$  to  $10^{-2}$  at t = 5 X  $10^4$ , but n decreases by about a factor of 2 at  $t = 10^6$  for the same thickness change. The importance of these relations between n and x and between n and t to Multivator is that the choice of the membrane thickness should not be arbitrary, but should be selected from considerations of the expected time available for the experiment and the steady-state condition. Clearly in fig. 6 if the time available for the experiment is, e.g.,  $10^6$  seconds a device using a  $10^{-2}$  cm membrane would be able to detect a factor of ten fewer microorganisms than the device using a  $10^{-3}$  cm membrane. If the time available for the experiment is, however, limited to, e.g., 10<sup>3</sup> seconds then use of a 10<sup>-2</sup> cm membrane would provide no increase in the detectability over a  $10^{-3}$  cm membrane even if the  $00_{2}$  steady-state conditions were satisfied; since it is not satisfied for the  $10^{-2}$  cm membrane the detectability would be less than that of the  $10^{-3}$  cm membrane. It can be seen from fig. 6 that the most efficient use of the separation device is when t and x just satisfy the steady-state condition, i.e. t equals 100 T.

The value of n can be found from figures 5 and 6 for different values of r since n is proportional to r, e.g. the values of n for r = 6 are equal to one tenth the values plotted in figures 5 and 6 for r = 60. For r = 60 the total dpm in the detection chamber exceeds 100 dpm for all of the curves in figures 5 and 6; not all the curves for r = 6, 30.

however, satisfy this condition.\* As an example of the applicability of figures 5 and 6 to systems having r values not equal to 60, consider the detectability estimate for teflon made earlier in this section where n equalled 20 - 30. From fig. 6 for r = 6,  $x = 10^{-2}$ cm and  $t = 7.2 \times 10^{4}$  seconds, n equals 38 which is quite good agreement considering that in the previous calculation  $x = 1.5 \times 10^{-2}$ cm and t was less than 1007.

One notes from equations (14) and (15) that for a given membrane system where one of the two solutes in the system is being produced at a constant rate any value of r can be achieved by the proper choice of x and t.

Let r = B = constant

then (14) can be written as:

(18) 
$$B = ax \left[ \frac{1}{t} - \frac{bx}{t} \left( 1 - e^{bx} \right) \right]$$

where a and b are constants.

Rearranging:

(19) 
$$ax - B = \frac{bax^2}{t} (1 - e^{\frac{-t}{bx}})$$

(20) 
$$1 - \frac{B}{ax} = \frac{bx}{t} (1 - e^{bx})$$
Choosing x so that, eg.,  $\frac{B}{ax} = 1/2$ 

then:

$$(21) \quad \frac{1}{2} \frac{t}{bx} = 1 - e^{\frac{-t}{bx}}$$

$$(22) \qquad 1 - \frac{t}{2bx} = e^{\frac{-t}{bx}}$$

\*The relationship between the total dpm in the detection chamber and r is given by equation (17).

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which is satisfied by choosing t so that:

(23) 
$$\frac{t}{bx} \approx 1.55$$

In practice, not all values of t and x are available so that not all values of r can be realized, however, in principle any r may be achieved for two such solutes. In the glucose- $CO_2$  case one notes from figures 5 and 6 that for silicone rubber and teflon the values of x and t are reasonable.

Comparing the values of n for the two membranes, silicone rubber and teflon, in figures 5 and 6, the teflon system has the lower n values. The detectability of the silicone rubber system ranges between a few ten thousand to a few hundred thousand bacteria, whereas the teflon system ranges between a few hundred to a few thousand bacteria. The teflon system is also not likely to violate the assumption made in the derivation of r(t,x,n) that the  $coldsymbol{$ 

In the actual life detection experiment one will have only information on the total cpm in the detection chamber at a given time, in addition to prior knowledge of the experimental system, e.g. membrane and device

That is, for the short experiments the steady-state condition requires that the membrane be very thin. If the membrane is thin enough more CO<sub>2</sub> will permeate the membrane than diffuses to the membrane and the concentration at the membrane will decrease until the two rates are equal.

32.

dimensions, etc. From this information alone one will not be able to determine m and n separately but only the product mn. This product can be related to the total cpm by using (14), (15) and (17), and converting the total number of dpm to the total number of cpm, W, by multiplying (17) by the counting efficiency for the system E, i.e.,

(24) 
$$(mn) = \frac{AK'C'}{x} \left[ \frac{W}{EHq'} - 6 \right] = \left[ \frac{W}{EHt} - \frac{6AK'C'}{x} \right]$$

$$\left[ 1 - \frac{Vx}{KAt} \left( 1 - e^{-\frac{KA}{Vx} t} \right) \right] = \left[ 1 - \frac{Vx}{KAt} \left( 1 - e^{-\frac{KA}{Vx} t} \right) \right]$$

For t large, (24) can be simplified to the approximation (r>>6):

(25) 
$$(mn) \sim \frac{W}{EHt}$$

or

(26) 
$$W \sim EH(mn)t$$

From (26) one notes that the slope of W versus t,  $\frac{dW}{dt}$ , is equal to EH(mn) and since H as a known constant, and E can be determined prior to the experiment, one can obtain (mn) from the slope  $\frac{dW}{dt}$  as t becomes large.

Rearranging (24) to give an expression for W:

(27) 
$$W = (mn) EH \left[t - \frac{Vx}{KA}(1-e^{-\frac{KA}{Vx}})\right] + \frac{6 AK'C'}{x} EHt$$
Expanding the exponential term in (27),

(28) 
$$W = (mn) EH \left[ t - \frac{Vx}{KA} \left( \frac{KAt}{Vx} - 1/2 \left( \frac{KAt}{Vx} \right)^2 + \frac{1}{6} \left( \frac{KAt}{Vx} \right)^3 - \cdots \right) \right] + \frac{6}{x} \frac{AK'C'}{x} EHt$$

For small t, W is given approximately by:

(29) 
$$W \sim (mn)$$
 EH  $\left[\frac{KA}{2Vx} t^2\right] + \frac{6 \text{ AK'C'}}{x}$  EHt  
The slope of W versus t,  $\frac{dW}{dt}$ , is approximately:

<sup>\*</sup>dpm = disintegrations per minute cpm = counts per minute

(30) 
$$\frac{dW}{dT} \sim (mn) \frac{EHKA}{Vx} t + \frac{6 AK'C'}{x} EH$$

so that (mn) can be found at small t using (30) from the slope of  $\frac{dW}{dt}$  versus  $t^*$ . For t equal to  $10^{\frac{1}{4}}$  seconds the error in the approximation (29) for a 5 x  $10^{-3}$  cm thick teflon membrane is less than 7%.

To summarize the section on constant populations we first found that the concentration of  $\mathrm{CO}_2$  in the upstream chamber approached an asymptote for large t (12). Therefore, the ratio of  $\mathrm{CO}_2$  to glucose molecules in the detection chamber,  $\mathrm{r}(\mathsf{t},\mathsf{x},\mathsf{n})$ , also approaches an asymptote at large t (14). This asymptote is proportional to the number of microorganisms, n, the rate of  $\mathrm{CO}_2$  production, m, and the membrane thickness x.

The detectable number of a given microorganism, e.g., E. Coli, decreases with increasing time t and with increasing membrane thickness x. For short times n is approximately independent of x, however, at long times n becomes inversely proportional to x (fig. 6). These relationships are important for maximizing the efficiency of Multivator in its role of life detection. In principle, at least, the possibility exists for achieving any desired ratio of one solute to another solute in the detection chamber by proper choices of t and x if one of the solutes is being produced at a constant rate. In the CO<sub>2</sub>-glucose case, we have adjusted the CO<sub>2</sub> rate by varying n so that x and t are reasonable. The corresponding detectability ranges between a few ten thousand to a few hundred thousand E. Coli for silicone rubber and between a few hundred to a few thousand E. Coli for teflon. Detectability estimates for other microorganisms can be found

<sup>\*</sup>Alternatively since C' is known, the slope of the plot  $\left(W - \frac{6AK'C'EHt}{x}\right)$  vs.  $t^2$  (rearrangement of (29)) can be used to determine mn. 34.

from figures 5 and 6 by replacing n by  $\frac{m^{i}n^{i}}{m}$  where n' is the number of microorganisms (m' is the known  $CO_{2}$  production rate for the microorganism on glucose) and n is the number of E. Coli (m is Clifton's (4) rate for  $CO_{2}$  production for E. Coli). For an unknown microorganism only the product of the  $CO_{2}$  production rate m and the number of microorganisms n can be obtained from the Multivator experiment. This product can be found using relations (26) and (30) for long and short experimental times respectively.

## C. Multiplying populations of microorganisms.

The discussion of the detectability estimates presented thus far has been limited to constant populations of microorganisms. There is good reason for this restriction if one is interested in investigating the intrinsic limitations of a given separation device. In the case of multiplying populations the detectability estimates depend strongly on the generation time of the microorganism. A short generation time exerts a levelling effect that tends to conceal the intrinsic limitations of a given membrane separation device, i.e., for a short generation time the device is capable of detecting a single microorganism.

It is important, however, to determine what information one can learn about amultiplying population using a membrane separation device and to illuminate the dependence of the detectability on the generation time. The general approach used in the section on constant populations for the derivation of r(t,x,n) will be used here except that n is now a function of t, i.e.,

(31) 
$$n = n_0 e^{t/g}$$
where  $g = generation time in seconds$ 
-693

 $n_0$  = the population at t = 0

The change in the number of molecules of CO<sub>2</sub> in a chamber of volume V during the infinitesimal time interval, dt, is:

(33) 
$$VC*(t) = mn_0 ge^{t/g} + constant$$
  
Evaluating the constant from the initial conditions:  
 $t = 0 \quad C = 0$ 

(34) 
$$C*(t) = \frac{mn_0 g}{v} (e^{t/g}-1)$$

The change in the number of molecules of  ${\rm CO}_2$  in a chamber of volume V having one surface a membrane of area A and of thickness x with a  ${\rm CO}_2$  permeability coefficient, K, during the infinitesimal time interval, dt, is:

(35) 
$$VdC = mn_0 e^{t/g} dt - \frac{KAC}{x} dt$$

$$\underline{AKt}$$

$$\underline{Multiplying by e}^{Vx} and rearranging:$$

(36) 
$$d(c e^{\frac{AKt}{xV}}) = \frac{mn_0}{V} e^{\left(\frac{t}{g} + \frac{AKt}{Vx}\right)} dt$$
Integrating:

(37) 
$$C(t)e^{Vx} = mn_0 \qquad gVx \qquad e^{\left(\frac{t}{g} + \frac{AKt}{Vx}\right)} + constant$$

Evaluating the constant from the initial conditions  $\frac{AKt}{Vx}$ :

(38) 
$$C(t) = \frac{mn_0gx}{(Vx + gAK)} \left( \frac{t}{e^g} - \frac{-AK}{Vx} t \right)$$

An expression for the ratio of  $CO_2$  to glucose in the detection chamber at time t for an initial population  $n_0$ ,  $r(t,x,n_0)$ , can be found from equations (12), (34), and (38).

(39) 
$$r(t,x,n_0) = \frac{c*(t) - c(t) v}{q^t}$$

$$= \frac{xmn_0 g(e^{t/g}-1) - \frac{mn_0 gx^2 v}{vx + qAK} (e^{t/g}-e^{-\frac{AKt}{vx}})}{AK^t C^t t}$$

In figure 7, the log of the initial population  $n_0$  that is detectable in an experiment continuing for time to is plotted versus log to for a  $10^{-2}$  cm thick silicone rubber membrane and for a  $10^{-3}$  cm thick teflon membrane. The constants used in the detectability calculations for a constant population were used in the calculations for a multiplying population. The value of g used was  $10^4$  seconds. From figure 7, one can see that when this greater than g both the silicone rubber system and the teflon system rapidly approach  $n_0 = 1$ . In the case of a multiplying population the detectability,  $n_0$ , becomes strongly dependent on the for the greater than g whereas in the constant population case, the detectability,  $n_0$ , is independent of that large the silicone case.

The total number of dpm in the detection chamber can be found from (17) where  $r(t,x,n_0)$  is substituted for r(t,x,n).

(40) total number of dpm = Hq' 
$$\left[ \Upsilon(t,x,n_0) + 6 \right]$$
  
The total number of cpm,  $\widetilde{W}$ , can be obtained from (40) by multiplying (40) by the counting efficiency of the system E, i.e.,

(42) 
$$\widetilde{W} = EH \left[ mn_{o}g \left( e^{t/g} - 1 \right) - \frac{\left( mn_{o}gxV \right)}{\left( Vx + gAK \right)} \left( e^{t/g} - e^{-Vx} \right) + \frac{6AK^{\dagger}C^{\dagger}t}{x} \right]$$
For t large, an approxmiate expression for  $\widetilde{W}$  is  $(\widetilde{r} \gg 6)$ :

(43) 
$$W = EH \left[ \frac{mn_0 g^2 AK}{(Vx + gAK)} e^{t/g} \right]$$

Taking the logarithms of (43):  
(44) In W 
$$\sim$$
 t/g + In  $\frac{\text{(EHmn g}^2 \text{ AK)}}{\text{(Vx + gAK)}}$ 

The plot of Inw versus t at large t is therefore linear with a slope of 1/g and a t=0 intercept of  $\ln\frac{(EHmn_0 g^2 AK)}{(Vx+gAK)}$ . Since all of the constants in the intercept are known except m,  $n_0$  and g, and g can be found from the slope, the product  $m_0$  can be calculated for an unknown population of microorganisms from the experimental data for t large. In the case where t is small, (42) is not readily simplified to an approximation in terms of m,  $n_0$  and g, however, in this case  $n_0$  n and the approximation (29)

should be valid.

To summarize the results of the multiplying population section, the upstream concentration of  ${\rm CO}_2$  does not approach an asymptote for large t as in the case of a constant population. Therefore,  $\tilde{r}$  also does not approach an asymptote for large t and the detectable number of microorganisms decreases with increasing time. The decrease is particularly sharp for t greater than g (fig. 7) and is approximately exponential for large t (43). As stated above, both the product  ${\rm mno}$  and g can be determined from the t large experimental data using (44). For t small the product  ${\rm mno}$  can be determined from the approximation found in the constant population section (29).

APPARENT INTEGRAL PERMEABILITY COEFFICIENT, K, AT 25 ± 2°C FOR C14 GLUCOSE THROUGH SILICONE RUBBER

TABLE !

	UPSTREAM CONCENTRATION MEMBRANE THICKNESS
1.27 2.62 4.43 11.40 14.57	7.7x105M h.6x105cpm/ml 8.0 ± .5 mil. time x 105 K x 102
1.9 1.9 8.1	'm1
. 22 1.60 2.00 2.45 3.94 6.32 7.16 8.03 8.03 9.90 11.21 11.21	7.7x103M 1.9x105cpm/ml 10.5 ± .5 mil* time x 105 K
16. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2	**************************************
.64 1.74 5.29 7.55 9.46 12.07 17.04 21.50	5.0X10 <sup>1</sup> M 1.1X10 <sup>6</sup> cpm 12.5 ± .5 1 time X 10 <sup>5</sup>
1.8	× 10 <sup>22</sup>
1.00 1.86 5.41 7.59 9.51 12.12 13.81 17.10 21.56	1.1x10 cpm/ml 13.5 ± .5 mil* time x 155 K x 162
18. 7.0 6.1 5.7	
.91	5.0x10 <sup>2</sup> M 1.1x10 <sup>6</sup> cpm/ml 13.0 ± .5 mil time x 10 <sup>5</sup> K x 10 <sup>2</sup>
15.	nl ii K × 1 <sup>12</sup>

<sup>\*</sup>Membrane presoaked in  ${
m H}_2{
m 0}$  prior to the start of the experiment for at least three days.

TABLE 11

APPARENT INTEGRAL PERMEABILITY COEFFICIENT, K, AT 25 ± 2°C FOR C14 GLUCOSE THROUGH 0.5 MIL. TEFLON (FEP) AND 4.0 mm POROUS GLASS (CORNING NO. 7930, 96% SiO2, 3% B2O3, PORE DIAMETER = 40A)

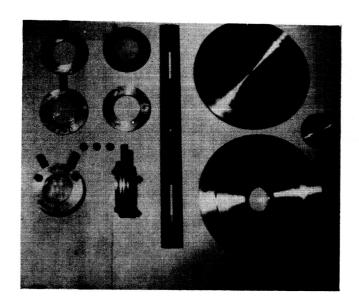
TEFLON  UPSTREAM CONC. = 7.8 X 103M		POROUS GLASS UPSTREAM CONC. = 1.4 X 10 M	
TIME X 105	к х 18 <sup>1</sup>	TIME X 103	к х 10 <sup>7</sup>
•77 sec	•7 cm <sup>2</sup> /sec	1.0 sec	2 8.1 cm /sec
1.58	2•5	1.5	11.
4.27	1.6	2.0	9•7
4.95	2.0	2.3	8.9
5•95	2.3	2.6	8.1
best	2.1	<b>3.</b> 0	7•3
straight line		3•5	6.5
		4.O	6.1
		4.5	5 <b>.</b> 6
		11.8	2•7
		23.6	2.0 .

TABLE III

DIFFUSION AND PERMEABILITY COEFFICIENTS FOR CO2 THROUGH VARIOUS MEDIA

MEDIUM	CONDITIONS	COEFFICIENT, cm <sup>2</sup> /sec
Silicone Rubber	H <sub>2</sub> 0-Membrane-H <sub>2</sub> 0 system; 25°C; upstream CO <sub>2</sub> conc. ~ 2X10 <sup>-3</sup> M	9 x 10 <sup>-6</sup>
Teflon	11	9 x 10 <sup>-8</sup>
Silicone Rubber <sup>5</sup>	Gas-Membrane-Gas system; room temp; pressure difference across the membrane = 60 cm Hg (3.5 X 10 <sup>2</sup> M)	3 x 10 <sup>-5</sup>
Teflon <sup>5</sup>	11	4 x 10 <sup>-7</sup>
Air 6	17.6°C; pressure ~ 1 atm.	1.63 x 10 <sup>-1*</sup>
Water <sup>7</sup>	18°c	1.46 x 10 <sup>-5</sup> *

Figure 1. Photograph of the 10cm<sup>2</sup> and 100cm<sup>2</sup> cells used for the Glucose Permeability Measurements



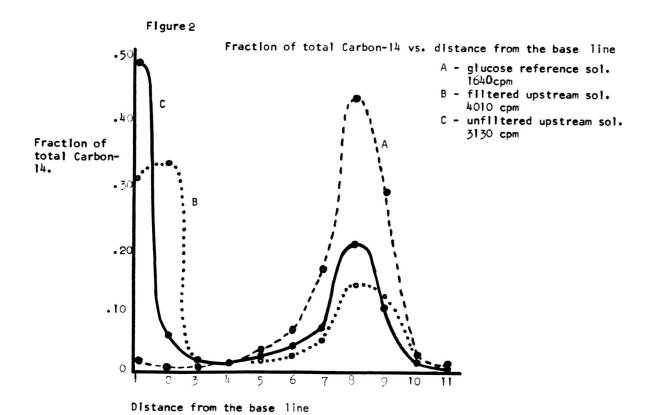
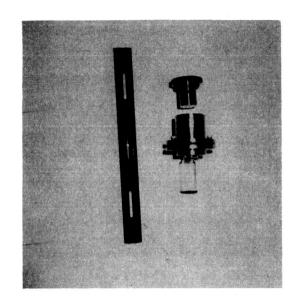
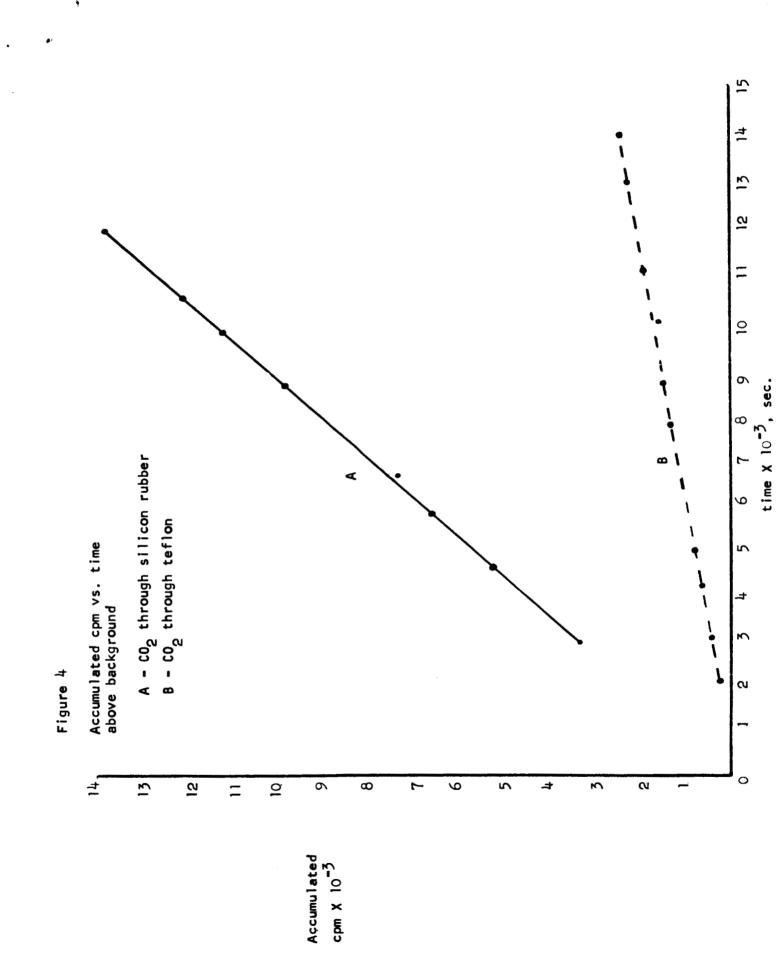
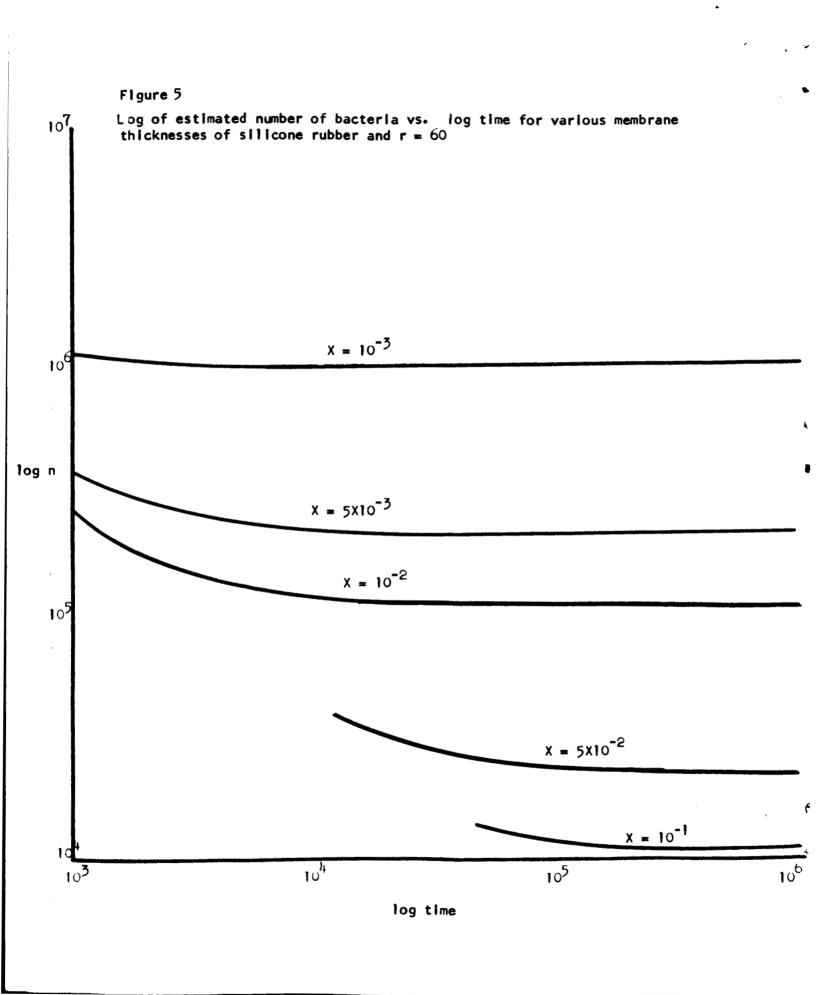
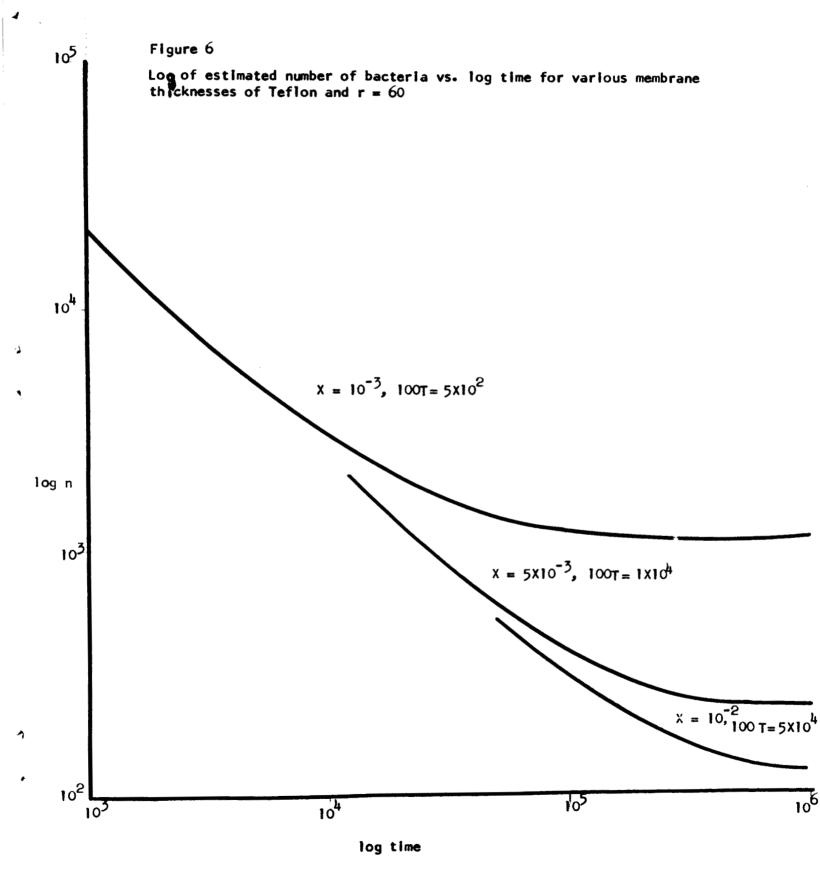


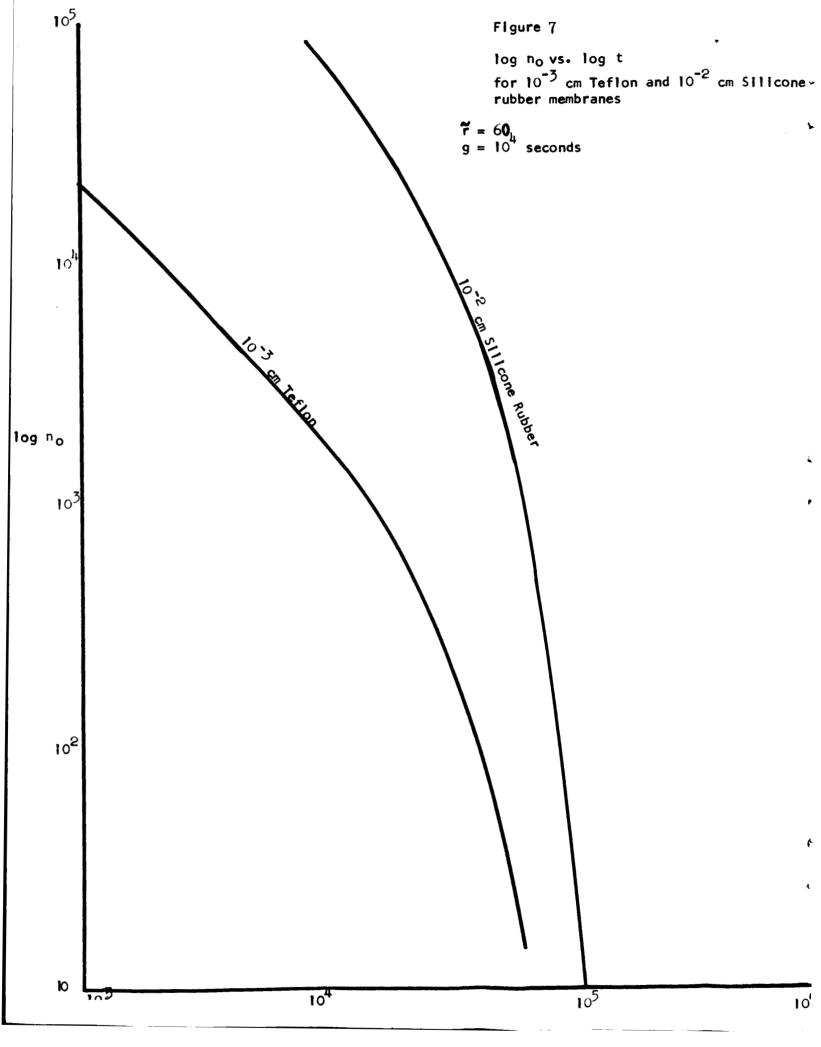
Figure 3. Cell used for  ${\rm CO_2}$  Permeability Measurements











## **Bibliography**

- 1. NASA Status Report covering the period March 1962 to April 1963 under NASA grant NsG 81-60, section 4.2 and appendix B.
- 2. Han, S., Tappi; 41, 540 (1958).
- 3. Shchennikova, M., Zhur. Priklad. Khim. 30, 838(1957).
- 4. Clifton, C., <u>Introduction to Bacterial Physiology</u>, McGraw-Hill, N.Y. (1957) p. 305.
- 5. Ruedi, B., Helv. Physiol. Acta. 20, 193(1962).
- 6. Jost, W., <u>Diffusion</u>, Acad. Press Inc., N.Y. (1952) p. 408.
- 7. Ibid. p. 475.